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85048 (US). **RENNERT, Jessica**; 8235 E. McDonald,
Scottsdale, AZ 85250 (US).

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(74) Agents: **GLENN, Michael** et al.; Glenn Patent Group,
3475 Edison Way, Ste. L., Menlo Park, CA 94025 (US).

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(71) Applicant: **INSTRUMENTATION METRICS, INC.**
[US/US]; 2085 Technology Circle, Suite 302, Tempe, AZ
85284 (US).

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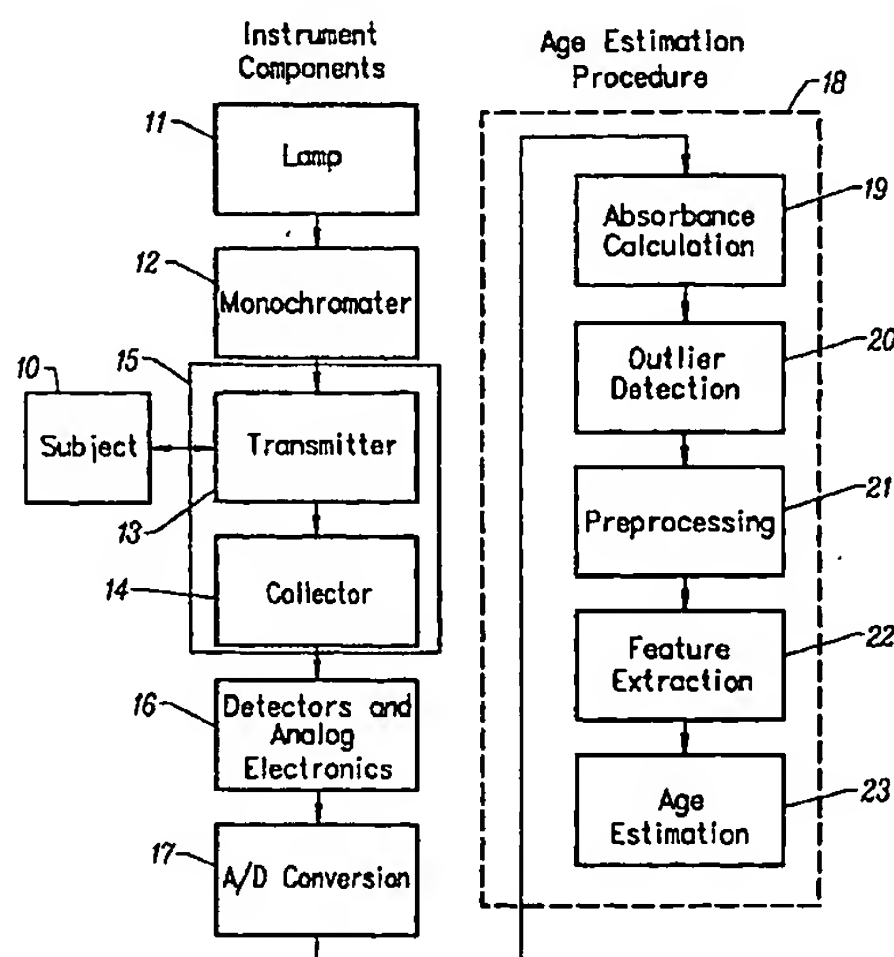
(72) Inventors: **RUCHTI, Timothy, L.**; 1501 West Sea Haze
Drive, Gilbert, AZ 85233 (US). **THENNADIL, Suresh**;
7250 S. Kyrene Road, Apt. 207, Tempe, AZ 85283 (US).
MALIN, Stephen, F.; 16228 S. 4th Street, Phoenix, AZ

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(54) Title: A SYSTEM FOR THE NONINVASIVE ESTIMATION OF RELATIVE AGE



(57) Abstract: Noninvasive instrumentation and procedures have been developed for estimating the apparent age of human and animal subjects based on the irradiation of skin tissue with near-infrared light. The method of age estimation provides additional information about primary sources of systematic tissue variability due to chronological factors and environmental exposure. Therefore, categorization of subjects on the basis of the estimated apparent age is suitable for further spectral analysis and the measurement of biological and chemical compounds, such as blood analytes. Furthermore, age determination of subjects has particular benefit in assessment of therapies used to reduce the effects of ageing in tissue and measurement of tissue damage.

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BACKGROUND OF THE INVENTION

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composition is based on detecting the magnitude of light attenuation resulting from its respective scattering and/or absorption properties.

The chronological age and type and duration of environmental exposure of skin tissue have a pronounced effect on the properties of tissue and is a
5 primary factor in tissue variability between individuals.

See, for example, W. Andrew, R. Behnke, T. Sato. *Changes with advancing age in the cell population of human dermis*, Gerontologia, vol. 10, pp. 1-19, (1964/65); W. Montagna, and K. Carlisle. *Structural changes in ageing*
10 *human skin*, The Journal of Investigative Dermatology, vol. 73, pp. 47-53, 1979. J. Brocklehurst, Textbook of Geriatric Medicine and Gerontology, Churchill Livingstone, Edinburgh and London, pp.593-623 (1973).

Therefore, NIR tissue spectroscopy can be used to detect, quantify, and
15 monitor age related effects in tissue through a noninvasive measurement process. Moreover, NIR tissue spectroscopy has particular benefit in several areas including estimation of blood analytes, assessment and monitoring of therapies used to reduce the effects of ageing in tissue and diagnosis and quantification of tissue damage.

20 Blood Analyte Prediction

While noninvasive prediction of blood analytes, such as blood glucose concentration, has been pursued through NIR spectroscopy, the reported success and product viability has been limited by the lack of a system for

compensating for structural variations between individuals that produce dramatic changes in the optical properties of the tissue sample

See, for example, O. Khalil. *Spectroscopic and clinical aspects of non-*
5 *invasive glucose measurements*, Clin Chem (1999) vol. 45, pp.165-77, and J.
Roe and B. Smoller, *Bloodless Glucose Measurements*, Critical Reviews in
Therapeutic Drug Carrier Systems, vol. 15, no. 3, pp. 199-241 (1998).

These differences are largely anatomical and provide distinct systematic
10 spectral absorbance features or patterns that can be related directly to
specific characteristics such as dermal thickness, protein levels, structure of
collagen bundles, dermal thinning, hydration, flattening of the epidermal-
dermal junction and thickness of the subcutaneous layer. While the
absorbance features are repeatable by subject over short periods of time,
15 over a population of subjects they produce confounding nonlinear spectral
variation. In addition, the changes of skin tissue of an individual as the result
of chronological ageing and/or environmental exposure lead to profound
differences in the volume of tissue sampled by the NIR measurement device.
Therefore, differences between subjects and within subjects over time are a
20 significant obstacle to the noninvasive measurement of blood analytes
through NIR spectral absorbance.

Previously, in the parent application to the current application, S. Malin and T.
Ruchti, *An Intelligent System For Noninvasive Blood Analyte Prediction*. U.S.
25 Patent Application Serial No. 09/359,191, filed July 22, 1999 an apparatus

and procedure for substantially reducing this problem by classifying subjects according to major skin tissue characteristics prior to blood analyte prediction was disclosed. The selected characteristics are representative of the properties of the actual tissue volume irradiated and the amount of the target
5 analyte that is sampled. By grouping individuals according to the similarity of spectral characteristics representing the tissue structure, the nonlinear variation described above is reduced and estimation of blood analytes becomes more accurate. Specifically, classification of NIR spectral data according to the apparent age or condition of the tissue will improve the
10 accuracy and robustness of models for estimating tissue/blood parameters, such as blood analytes, through the significant reduction of sample variability without the addition of other measurement devices (see S. Malin and T. Ruchti, *An Intelligent System For Noninvasive Blood Analyte Prediction*, U.S. Patent Application Serial No. 09/359,191, filed July 22, 1999).

15 Apparent Ageing of Skin Tissue

The effects of ageing on skin tissue include two separate phenomena: chronological and photo ageing. Chronological ageing is typified by natural changes in the skin over time, such as dermal thinning, changes in level of
20 hydration, flattening of the epidermal-dermal junction and reduced sebum/sweat production.

For example, see A. Oikarinen, *Ageing of the skin connective tissue: how to measure the biochemical and mechanical properties of ageing dermis*,
25 *Photodermatology Photoimmunology & Photomedicine* (1994) vol. 10, pp. 47-52; N. Fenske, and C. Lober, *Structural and functional changes of normal*

ageing skin, J Am Acad Dermatol (1996) vol. 15, pp. 571-583; M. Gniadecka, and G. Jemec, *Quantitative evaluation of chronological ageing and photo ageing in vivo: studies on skin echogenicity and thickness*, Br J Dermatol (1998) vol. 139, pp. 815-821.

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Photo ageing is an alteration or damaging of skin as a result of sun exposure, manifested by dryness, solar elastosis, irregular pigmentation and fine wrinkling, and is the cause of premature ageing of skin. See, for example R. Stern, *The Measure of Youth*, Arch Dermatol (1992) vol. 128, pp. 390-393.

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Ultrasound has been used to reveal that changes in the upper dermis are related to photo ageing and changes in the lower dermis are related to chronological ageing. See A. Oikarinen, *Ageing of the skin connective tissue: how to measure the biochemical and mechanical properties of ageing dermis*,

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Photodermatology Photoimmunology & Photomedicine (1994) vol. 10, pp. 47-52. The upper dermis becomes thicker (solar elastosis) with increased sun exposure and the lower dermis degrades with chronological age. See J. Rigal, C. Escoffier, B. Querleux, B. Faivre, P. Agache, J. Leveque, *Assessment of Ageing of the Human Skin by In Vivo Ultrasonic Imaging*, Society for

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Investigative Dermatology (1989) vol. 93, pp. 621-625.

As a result of societal pressure for tanned young skin, pharmaceutical and cosmetic companies have been developing and marketing products that claim to repair the effects of photo-damage to skin and restore skin to its youthful condition. The ability to quantitatively measure the apparent age or condition of tissue is useful in determining the effectiveness of topical drugs used to

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reverse damage due to photo ageing . See R. Stern , *The Measure of Youth*, Arch Dermatol, (1992), vol. 128, pp. 390-393. An *in vivo*, quantitative technique would be of great benefit in assessing the effectiveness of treatments for photo-damaged tissue. See M. Quan, C. Edwards, and R.
5 Marks, *Non-invasive In Vivo Techniques to Differentiate Photodamage and Ageing in Human Skin*, Acta Derm Venereol, vol. 77, pp. 416-419 (1997).

However, no technique has been reported for quantitatively determining apparent age on the basis of a noninvasive measurement. Existing methods
10 for age determination generally rely on invasive procedures or subjective evaluation. The amount of photo-damage and the effects of age-reversing drugs are typically determined by visual inspection of the skin by a trained individual and subsequent assignment of a grade representing the degree of damage. Several groups have proposed methods using standardized
15 photographs representation the different degrees of sun damage in an attempt to standardize the age ratings. These methods have been found to be subjective and not repeatable. Furthermore, these qualitative methods only provide a surface measurement of the effectiveness of the product being tested and do not provide any information about true structural or chemical
20 changes in the tissue. See R. Stern, *The measure of Youth*, Arch Dermatol, vol. 128, pp. 390-393 (1992).

SUMMARY OF THE INVENTION

25 The present invention provides a method and apparatus for non-invasively determining the apparent age tissue, *in vivo*. A spectroscopic apparatus in

conjunction with an optical interface is used to measure tissue properties and characteristics that are manifested spectrally and that vary systematically according to the subject's age and environmental exposure. A novel method is disclosed that uses *in vivo*, non-invasive NIR measurements to estimate the
5 apparent age of the skin and/or classify the skin according to predefined age group categories.

The procedure for age estimation employs a calibration model that is empirically derived from a set of exemplary samples consisting of NIR tissue
10 measurements and the actual chronological age of a population of subjects. The model is a set of parameters and computer generated code that is implemented to estimate the subject's age. The estimation consists of an actual age determination in years and one or more relative property magnitudes that reveal information regarding the tissue properties of the
15 sampled tissue volume.

The apparent age estimate provides a reliable and repeatable quantitative measure of the condition of the skin with respect to the combined effects of chronological ageing and photo ageing. In addition, magnitude of the two
20 types of ageing is deduced from NIR measurements by targeting specific tissue volumes and/or decomposing the measurements through multivariate factor analysis to reveal underlying variation correlated to specific ageing related tissue parameters such as dermal thickness and hydration. The resulting age estimation and/or classification is also suitable for categorization
25 of spectral data prior to blood analyte prediction.

Figure 9 is a plot of the mean second derivative spectra of the separate age categories of Figure 8 according to the invention;

- 5 Figure 10 is a plot of the mean spectra of the separate age categories of Figure 8 according to the invention;

Figure 11 is a scatter plot showing the separation of principle component scores associated with young and old subjects according to the invention; and

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Figure 12 is a plot of a membership function for a fuzzy classification of subjects by age according to the invention.

DETAILED DESCRIPTION

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The current invention provides an apparatus for measuring the infrared absorption by tissue irradiated with near-infrared energy, a procedure for estimating the subject's age and a procedure for classifying the subject into age related categories for further spectral analysis and blood analyte

20 prediction.

APPARATUS

The apparatus includes an energy source, a sensor element, an interface 15 to the subject 10, a wavelength selection device and an analyzer. The energy

25 source generates and transmits near-infrared energy in the wavelength range 700-2500 nanometers and consists of a device such as an LED array or a

quartz halogen lamp. The method of wavelength separation includes a monochromator, an interferometer or successive illumination through the elements of an LED array. The optical interface 15 includes a means for transmitting energy 13 from the source to the target skin tissue measurement site; for example, a light pipe, a fiber-optic probe, a lens system or a light directing mirror system, and a means for collecting energy 14 from the target site. Energy is collected from the surrounding tissue areas in reflectance mode at an optimally determined distance(s) through the use of staring detectors or fiber-optic probes. Alternately, energy is collected in transmission mode through a skin flap, ear lobe, finger or other extremity. The sensing elements are detectors that are responsive to the targeted wavelengths. The collected light is converted to a voltage and sampled through an analog-to-digital converter for analysis on a microprocessor-based system.

15

A block diagram of the integrated system is shown in Figure 1. In the preferred embodiment the instrument employs a quartz halogen lamp 11, a monochromator 12 and InGaAs detectors 16. The detected intensity from the sample is converted to a voltage through analog electronics 16 and digitized through a 16-bit A/D converter 17. The spectrum is passed to the age estimation procedure for processing. First, the absorbance is calculated on the basis of the detected light through $-\log(R/R_0)$ where R is the reflected light and R_0 is the light incident on the sample determined by scanning a reference standard. For example, Figure 2 shows a typical absorbance spectrum collected on an apparatus according to the preferred embodiment.

Subsequent processing steps, described below, result in either an apparent age estimate 23 or an apparent age classification 41.

Alternately, the measurement can be accomplished with existing NIR spectrometers that are commercially available including a Perstorp Analytical NIRS 5000 spectrometer or a Nicolet Magna-IR 760 spectrometer. In addition, the measurement can be made by collecting reflected light off the surface of the skin or light transmitted through a portion of the skin, such as the finger or the ear lobe. Further, the use of reflectance or transmittance can replace the preferred absorbance measurement.

Optical Interface

The specific tissue layer sampled is related to the type of ageing. For example, degradation of the upper dermis is related to photo ageing while chronological ageing is distinguished by changes in the lower dermis. See, for example M. Gniadecka and G. Jemec, *Quantitative evaluation of chronological ageing and photo ageing in vivo: studies on skin echogenicity and thickness*, Br J Dermatol (1998) vol. 139, pp. 815-821.

The upper dermis becomes thicker (solar elastosis) with increased sun exposure and the lower dermis degrades with chronological age. In the present invention the specific tissue layer that is sampled is controlled through the spacing of the point of illumination and the point of light detection by the optical interface (15). The point of illumination is set through a focusing lens or delivered directly via a fiber optic probe. The point of detection is controlled through starrng optics or a fiber optic probe. The preferred spacing

for the determination of photo ageing is less than 300 μm . The preferred source to illumination spacing for chronological ageing is 0.3 to 3 mm based on the wavelength region (0.3 mm between 2000 and 2500 nm, 1-2 mm in the 1500-1850 nm range and 3 mm in the 700-1400 nm range).

5 General Age Estimation Procedure

The general procedure for age estimation based on the measured NIR spectrum, shown in Figure 3, is implemented in a microprocessor 18 that automatically receives the measurement information from the ADC 17. The
 10 procedure for age estimation includes the sub-procedures outlier detection 20, preprocessing 21 and estimation 23. Each sub-procedure is performed on the basis of a calibration set of exemplary measurements that includes an absorbance spectrum and a measure of the apparent age (or chronological age or photo-age) of the subject population. Presented below is an overview
 15 of the procedure for estimating the apparent age on the basis of spectral measurements. Further details are provided in the subsequent Implementation section, below.

Measurement

20 The measurement 30 is a spectrum denoted by the vector $m \in \mathbb{R}^N$ of absorbance values pertaining to a set of N wavelengths $\lambda \in \mathbb{R}^N$ that span the near infrared (700 to 2500nm). A typical plot of m versus λ is shown in Figure 2.

Outlier Detection

The outlier detection procedure 20 is a method for detecting invalid measurements due to spectral variations resulting from problems in the instrument, poor sampling of the subject or a subject lying outside the calibration set. The preferred method for the detection of spectral outliers is through a principal component analysis and a further analysis of the resulting residuals. First, the spectrum m is projected onto five eigenvectors, contained in the matrix o , that were previously developed through a principal component analysis (on a calibration set of exemplary absorbance spectra) and are stored in the computer system of the device. The calculation is given by

$$xpc_o = \sum_{k=1}^5 mo_k, \quad (1)$$

and produces the 1 by 5 vector of scores, xpc_o where o_k is the k^{th} column of the matrix o . The residual q is determined according to

$$q = m - xpc_o o^T \quad (2)$$

and compared to three times the standard deviation of the expected residual (of the calibration set). If greater, the sample is reported to be an outlier and the age determination procedure is terminated.

20

Preprocessing

The optional step of preprocessing 21 includes operations such as wavelength selection, scaling, normalization, smoothing, derivatives, filtering

and other transformations that attenuate the noise and instrumental variation without affecting the signal of interest. The preprocessed measurement, $x \in \mathbb{R}^N$, is determined according to

(3)

5 where $h: \mathbb{R}^{N \times 2} \rightarrow \mathbb{R}^N$ is the preprocessing function. Wavelength selection is performed on the data to eliminate extraneous variables that may bias the calibration or portions of the measured spectrum with a low signal-to-noise ratio. This is performed visually and through an analysis of the noise at each wavelength. The specific preprocessing methods used for age estimation
 10 may include wavelength selection, multiplicative scatter correction and derivatives. See, for example P. Geladi, D. McDougall and H. Martens. *Linearization and Scatter-Correction for Near-Infrared Reflectance Spectra of Meat*, Applied Spectroscopy (1985) vol. 39, pp. 491-500, and A. Savitzky and M. Golay. *Smoothing and Differentiation of Data by Simplified Least Squares*
 15 *Procedures*, Anal. Chem., vol. 36, no. 8, pp. 1627-1639 (1964).

Estimation

The estimation procedure employs a calibration model 31 that maps the
 20 preprocessed spectrum through a linear or nonlinear mapping to an estimate of the age. In the linear case, given the processed spectrum x , and the

$$\hat{y} = \sum_{k=1}^N w_{c,k} x_k$$

calibration model coefficients w_c , the age estimate is determined according to

(4)

were $w_{c,k}$ is the k^{th} element of w_c and \hat{y} is the age estimate. One skilled in the art will appreciate that a nonlinear mapping from x to \hat{y} can also be easily specified through artificial neural networks, nonlinear partial-least squares regression or other nonlinear method of calibration.

5

See, for example H. Martens and T. Naes, Multivariate Calibration. New York: John Wiley and Sons, (1989) pp. 419; P. Geladi, D. McDougall and H. Martens. *Linearization and Scatter-Correction for Near-Infrared Reflectance Spectra of Meat*, Applied Spectroscopy, (1985) vol. 39 pp. 491-500; and Y. Pao, Adaptive Pattern Recognition and Neural Networks, Addison-Wesley Publishing Company, Inc., Reading, MA, (1989).

The preferred model is linear and is constructed through factor analysis to decompose the high dimensional (redundant) data consisting of absorbance, intensity or reflectance measurements at several hundred wavelengths to a few significant factors that represent the majority of the variation within the data set. The factors that capture variation in the spectra that correlate with age are used in the calibration model and the samples are projected into the resulting factor space to produce a set of scores for each sample. Finally, multiple linear regression is applied to model the relationship between the scores of the significant factors and the apparent age 23 of the subject.

General Age Classification Procedure

NIR measurements 30 from tissue samples of varying age can be classified into age groups according to their physical and chemical properties. The procedure for classifying samples according to predefined age groups includes preprocessing 21 the data for feature enhancement, performing a

factor analysis for variable reduction and developing a classification calibration on the significant factors. The classification calibration is any mathematical or statistical technique, such as Fisher's Linear Discriminant Analysis, that assigns a label to a sample and, using a decision rule, can
5 determine the population membership of the sample. Methods, such as multiplicative scatter correction and derivatives, are used to enhance features associated with age parameters.

See R. Duda and P.E. Hart, Pattern Classification and Scene Analysis, John
10 Wiley and Sons, New York, 1973. Also see P. Geladi, D. McDougall and H. Martens. *Linearization and Scatter-Correction for Near-Infrared Reflectance Spectra of Meat*, Applied Spectroscopy, (1985) vol. 39, pp. 491-500.

A factor-based analytical method, such as principal component analysis
15 (PCA), is applied to the preprocessed data in order to reduce the data set down to a few significant age-related factors. The classification calibration is developed using the significant age-related factors. The classification calibration can then be used to determine the membership of a new sample. Figure 4 is a flow diagram of the basic age classification steps. The general
20 procedure is implemented in a microprocessor 18 that automatically receives the measurement information from the ADC.

Outlier Detection and Preprocessing

25 The procedures for outlier detection 20 and preprocessing 21 are similar to those defined in the General Age Estimation Section. The specific procedures are

optimized on the basis of the calibration set for feature extraction 22 and classification 41 as presented in the Implementation Section, below.

Feature Extraction

5 Feature extraction 22 determines the salient characteristics of measurements that are relevant for age classification. Feature extraction 22 is any mathematical transformation that enhances a quality or aspect of the sample measurement for interpretation. The purpose of feature extraction 22 is to concisely represent and enhance the properties and characteristics of the
10 tissue measurement site for age classification. In addition, the features provide significant information about the tissue properties they represent and can be used for alternate purposes such as system diagnostics or optimization.

15 The features are represented in a vector, $z \in \mathcal{R}^M$ that is determined from the preprocessed measurement through

$$z = f(\lambda, x) \quad (5)$$

where $f: \mathcal{R}^N \rightarrow \mathcal{R}^M$ is a mapping from the measurement space to the feature space. Decomposing $f(\bullet)$ yields specific transformations, $f_i(\bullet): \mathcal{R}^N \rightarrow \mathcal{R}^{M_i}$ for
20 determining a specific feature. The dimension, M_i , indicates whether the i^{th} feature is a scalar or a vector, and the aggregation of all features is the vector z . When a feature is represented as a vector or a pattern, it exhibits a certain structure indicative of an underlying physical phenomenon.

25 The individual features are divided into two categories:

abstract, and

simple.

Abstract features do not necessarily have a specific interpretation related to the physical system. Specifically, the scores of a principal component
5 analysis are useful features although their physical interpretation is not always known. (See H. Martens, T. Naes, Multivariate Calibration, John Wiley and Sons, New York (1989) pp. 419. For example, the utility of the principal component analysis is related to the nature of the tissue absorbance spectrum. The most significant variation is generally related to the tissue
10 structure, which varies systematically with age. Therefore, the scores from the principal component analysis constitute a valuable set of features in that they provide information that can be used for age determination.

According to a preferred realization of the invention, the use of principal
15 component analysis to represent spectral variation related to age is demonstrated through the Experimental Data Set, more fully described in the Implementation Section, below. The set of spectra, collected on 266 subjects of diverse age and sex, was subjected to principal component analysis in two wavelength regions: Region 1 (1100-1380 nm) and Region 2 (1550-1800
20 nm). The scores of selected principal components were plotted versus one another as shown in Figures 5a, 5b and 5c, with different symbols for data points corresponding to individuals above (old) and below (young) the mean age of 49 years. The scores, representing variation in the spectra, show a pronounced systematic separation according to subject's age. The
25 unmistakable grouping of the data according to age in the scatter plots of Figure 5 clearly demonstrates the utility of feature extraction through principal

component analysis and the possibility of subsequent classification of the subjects according to age. In addition, the correlation between age and the lower numbered principal components indicates that age represents a primary source of variation in the spectra.

5

Simple features are derived from an a priori understanding of the sample and can be related directly to a physical phenomenon. For example, the thickness of the dermis varies systematically with age and results in specific spectral manifestations. These spectral variations are extracted and enhanced and
10 serve both as a feature for age classification and as a measurement of their respective tissue properties.

Although the full spectrum can be passed to the classification model 40 for age classification 41, a preferred realization of the invention employs either of
15 two specific methods of feature extraction that provide superior classification performance and measurement of other relevant tissue properties:

the scores from factor analysis, and

the estimates from partial least squares (PLS) regression.

The detailed implementation of the procedure for extracting these features on
20 the basis of a calibration set is provided in the next section, below.

Crisp Classification

The classification 41 of the subject's age on the basis of the extracted
25 features is performed through a classification step that involves a mapping and a decision. The mapping step is given by

$$L = f(z) \quad (6)$$

where L is a scalar that can be used to measure the distance from the predefined age categories. For example, two values, L_{old} and L_{young} , associated with the representative or mean value of L for the “old” and “young” categories respectively are predefined, and the class assignment is based on the closeness of L to L_{old} and L_{young} . For example, the distance of L from a previously defined class boundary means that classes can be measured by

$$\begin{aligned} d_{old} &= |L_{old} - L| \\ d_{young} &= |L_{young} - L| \end{aligned} \quad (7)$$

10 The decision is made as follows:

if $d_{old} < d_{young}$ then the apparent age of the tissue is classified as old,

if $d_{old} > d_{young}$ then the apparent age of the tissue is classified as young.

The mapping and decision limits are determined from a calibration set of exemplary features and corresponding apparent age reference values through a classification calibration procedure. Commonly known methods of classification calibration include linear Discriminant analysis, SIMCA, k nearest-neighbor, fuzzy classification and various forms of artificial neural networks. Furthermore, one skilled in the art will appreciate that more than two distinct classes for age can be defined with an upper limit based on the accuracy of the measurement device.

See, for example R. Duda and P. Hart, Pattern Classification and Scene Analysis, John Wiley and Sons, New York, (1973); S. Wold and M. Sjostrom. *SIMCA: A method for analyzing chemical data in terms of similarity and*

analogy, Chemometrics: Theory and Application, ed. B.R. Kowalski, ACS Symposium Series, vol. 52, (1977); J. Bezdek and S. Pal, eds., Fuzzy Models for Pattern Recognition, IEEE Press, Piscataway, NJ, (1992); J. Keller, M. Gray and J. Givens. *A Fuzzy K nearest Neighbor Algorithm*, IEEE Transactions on Systems, Man, and Cybernetics, Vol. SMC-15, No. 4, pp. 580-585, (July/August, 1985); and Y. Pao, Adaptive Pattern Recognition and Neural Networks, Addison-Wesley Publishing Company, Inc., Reading, MA, (1989).

Fuzzy Classification

10

While statistically based class definitions provide a set of classes applicable to age classification, the apparent age of a tissue sample and the resulting spectral variation change over a continuum of values. Consequently, the natural variation in the spectra results in class overlap. Distinct class boundaries based on age do not exist and many measurements are likely to fall between classes and have a statistically equal chance of membership in any of several classes. Hence, "hard" class boundaries and mutually exclusive membership functions may be insufficient to model the variation found in a target population.

20

A more versatile method of class assignment is based on fuzzy set theory. (See J. Bezdek, and S. Pal, eds., Fuzzy Models for Pattern Recognition, IEEE Press, Piscataway, NJ, (1992); C. Chen, ed., Fuzzy Logic and Neural Network Handbook, IEEE Press, Piscataway, NJ (1996); and L. Zadeh, *Fuzzy Sets*, Inform. Control, vol. 8, pp. 338-353, (1965).

25

Generally, membership in fuzzy sets is defined by a continuum of grades and a set of membership functions that map the feature space into the interval [0,1] for each class. The assigned membership grade represents the degree of class membership with "1" corresponding to the highest degree. Thus, a
 5 sample can simultaneously be a member of more than one class.

The mapping from feature space to a vector of class memberships is given by

$$c_k = f_k(z) \quad , \quad (8)$$

where $k=1,2,\dots,P$, $f_k(\bullet)$ is the membership function of the k^{th} class, $c_k \in [0,1]$ for
 10 all k and the vector $c \in \mathbb{R}^P$ is the set of class memberships. An example of the general equation employed to represent a membership function is

$$y = e^{\frac{-1}{2\sigma^2}(z-\bar{z})^2} \quad , \quad (9)$$

where y is the degree of membership in a sub-set, z is the feature used to determine membership, \bar{z} is the mean or center of the fuzzy sub-set and σ is
 15 the standard deviation. However, one skilled in the art will appreciate that the suitable membership function is specific to the application.

The membership vector provides the degree of membership in each of the predefined classes and can be used for blood analyte prediction as disclosed
 20 in the parent application to the current application, S. Malin, T. Ruchti, *An Intelligent System For Noninvasive Blood Analyte Prediction*, U.S. Patent Application Serial No. 09/359,191, (July 22, 1999). Alternately, the degree of class membership can be used to calculate the apparent age, photo-age or

chronological age of an individual with a suitable function for defuzzification. The defuzzification function can be determined as described by Bezdek, *et al.* (See J. Bezdek, and S. Pal, eds., Fuzzy Models for Pattern Recognition, IEEE Press, Piscataway, NJ, (1992). Also see the parent application to the
5 current application, as above.) Alternately, a calibration set of exemplary spectral measurements and associated age reference values can be used to determine a calibration model for mapping the class membership to an estimate of the selected age.

Implementation Details

10 Various realizations of the invention, comprising specific procedures for age estimation and classification are described in detail below.

Experimental Data Set

15 A study was performed to generate calibration and validation data for the procedures subsequently described. Two Hundred sixty-six human subjects of diverse age, sex and ethnicity were recruited at a local health care facility, and detailed demographic information about each participant was recorded. Four replicate absorbance spectra were measured on each subject's forearm
20 with the previously described spectrometer apparatus according to the preferred embodiment. One sample per each participant was included in the data set. Henceforth, the total set of spectra and demographic information shall be referred to as the "Experimental Data Set."

25 While this is a specific experiment aimed at the determination of a suitable set for calibrating the age determination apparatus, one will readily appreciate

that for different subjects and for different target performance levels other experiments with smaller or larger subject populations would be performed. Moreover, experiments specific to photo ageing versus chronological ageing would replace this experiment given a different target set of age estimates.

5 Estimation

Two implementations of the age estimation procedure are described in the following sub-sections differing by the wavelength region of the absorbance spectrum applied.

10

Age Estimation 1 (1100-1400 nm)

In the first implementation, the procedure outlined in Figure 3 was employed. The (PCA q-residual) outlier analysis was performed as described above and
15 36 samples were removed due to unusually high residuals. No preprocessing was applied and the wavelength region was limited to 1100-1400 nm to ensure sampling of the lower dermis. While sampling of the subcutaneous tissue also occurs in this wavelength region, the magnitude of the fat absorption features is indicative of the absorption characteristics of the dermis
20 and epidermis and provides an indirect measurement of the target absorption characteristics. Partial least squares (PLS) regression with 17 factors was applied to the entire data set to develop a calibration model. (See P. Geladi and B. Kowalski, *Partial least-squares regression: a tutorial*, Analytica Chimica Acta, vol. 85, pp. 1-17 (1986).

25

The performance of the estimation model was evaluated through cross-validation using a "leave-one-out" strategy and calculating the standard error values. The cross-validation procedure was used iteratively to estimate the age of each sample by using all other samples to construct the calibration
5 model. After each sample had been predicted the standard error of cross-validation (SECV) was computed as the root mean square error of the cross-validation age estimates. The results of the estimated chronological age (through cross-validation) versus the actual age are shown in Figure 6. The standard error of prediction is 7.8 years and the plot shows a clear statistically
10 significant level of estimation. The error in prediction may be attributed to two phenomena. First, the rate of chronological ageing in different individuals is not necessarily the same. As a result, the reference values contain error by definition. Secondly, photo-damage to the upper dermis represents a confounding effect that may limit the accuracy of the estimation model.

15 *Age Estimation 2 (1500-2400 nm)*

In this implementation the estimation of age 23 is performed using partial least squares (PLS) regression on upper wavelength regions of the absorbance spectra. While the lower wavelength region used in Age Estimation Method
20 One targets primarily the dermis and subcutaneous tissue, the high absorbance of water prevents significant sampling of the subcutaneous tissue at upper wavelengths. Since age related parameters manifest themselves most distinctly in the dermis and epidermis of the tissue, limiting the wavelength range to the 1500 to 2400 nm region limits potential interference:
25 contributed by the subcutaneous tissue.

Outlier analysis was applied to the data set as previously described. The remaining samples were split into calibration (60%) and test (40%) sets. No feature enhancement or preprocessing techniques were applied and cross-validation was used on the calibration set to determine that eight was the
5 optimal number of factors for feature extraction. The calibration was then developed using the calibration set and applied to the test set for validation.

The test set had a standard error of prediction of 8.0 years. The cause of the error in the prediction can be explained similarly to that for Age Estimation
10 Method One: a disparity between the actual chronological age of the subject and the apparent age of the tissue; consequently, a subject's skin condition may resemble that of the skin of a person several years older due to photo ageing effects. Thus, in a person using a photo-damage-reversing drug, periodically predicting the apparent age of the skin throughout the course of
15 treatment would be an effective way to monitor the performance of the drug.

Classification

The classification 41 of subjects according to age was implemented using
20 three different approaches. The first two involve a crisp classification system in which distinct class boundaries are defined and each sampled absorbance spectrum has membership in only one class. The third method involves the use of a fuzzy system to arrive at an estimate of the degree of membership in each of several predefined classes for each sample.

25

In the application of this system to the prediction of blood analytes, the accuracy of chronological age prediction is not the most important element but rather the characterization of the apparent age of the sampled tissue volume. Nevertheless, one skilled in the art will appreciate that the following methods
5 and procedures are easily adapted to other applications.

Age Classification 1 (Crisp 1100-1800 nm)

The first age classification implementation is depicted in Figure 7 and involves
10 the prediction of the subject's age using a linear model developed through partial least squares regression. In the current implementation, the wavelength range of the spectrum 30 is truncated to the regions 1100-1380 nm and 1550-1800 nm in the wavelength selection process 50. Next, the subject's age is predicted through a calibration model. The model, developed
15 through PLS on a calibration set of exemplary samples, consists of a set of coefficients contained in the vector w 51 and is applied as shown in Figure 7 to produce the age prediction, a 52. The subject is classified as "young" 54 or "old" 53 by comparing a 52 to the mean age $\bar{a} = 49$ as detailed in the figure. One skilled in the art will appreciate that more than two distinct classes can be
20 defined with an upper limit based on the accuracy of the measurement device.

Using the Experimental Data Set, the PLS calibration model was developed using 20 factors. The classification accuracy was evaluated through cross-validation in which groups of 12 samples were iteratively left out of the
25 calibration model. The accuracy of age classification was found to be 79%. The reason for the error is attributable to the continuum of chronological ages and skin tissue properties that were measured using a classification with crisp

boundaries. In Age Classification 2 the boundary is modified by removing samples from the calibration that represent chronological ages in between the two main old and young categories.

5 *Age Classification 2 (Crisp 1500-2500 nm)*

The second age classification procedure is detailed in Figure 8 and was developed based on the calibration set described in the Age Estimation 2 Section. The procedure provides two groups: a young group that includes
10 ages from 20 to 39 years old and an old group which ranged from 60 to 84 years old. The middle age range was left out in order to get a more distinct separation between age groups. A 33-point second Savitzky-Golay derivative
15 60 (see A. Savitzky and M. Golay. *Smoothing and Differentiation of Data by Simplified Least Squares Procedures*, Anal. Chem., vol. 36, no. 8, pp. 1627-
1639, (1964). was applied to the spectral data 30 to enhance the variation in the mean spectra 70, 71 due to age (see Figure 9). Referring now to Figure
20 10, the largest separation between the mean spectra 70, 71 of the two age groups was observed over the wavelength regions of 1500 to 2500nm. Thus, this was the wavelength region selected 51 for further analysis. A principal
25 component analysis (PCA) was applied to the spectral data 30 over the selected wavelength region. The scores from the PCA were plotted against one another to reveal clustering of the data related to age. As shown in Figure 11, two clusters corresponding to the two age groups formed when the scores from principal component one was plotted against scores from
principal component four 61. These two groups can be used to represent tissue with young and old characteristics. Using these two distinct age groups

a classification model was developed from the age significant scores and used to classify the apparent age of new samples.

The procedure of Figure 8 uses two eigenvectors associated with principal components 1 and 4 to determine the scores, xpc_1 and xpc_2 , of each sample. A Discriminant function is applied to classify the subjects based on the two features contained in m through the equation shown in the figure to produce the scalar, L 63. This result is compared to \bar{L} 64, the center between the two classes. If $L > \bar{L}$ then the subject is classified as a old 65. If not, the spectrum is classified as belonging to the young class 66. Using the calibration set, the linear mapping 62 of Figure 8 was developed via linear Discriminant analysis (see Duda, R.O. and P.E. Hart, *Pattern Classification and Scene Analysis*, John Wiley and Sons, New York, 1973) and produced the following weight vector

$$w = [0.1198 \quad 0.9928] \quad (10)$$

From the calibration set, the mean value for L was found to be 0.1131. Using these parameters in conjunction with the procedure of Figure 8 allowed age prediction in the test set with 90% accuracy.

20 Age Classification 3 (Fuzzy)

The final procedure for age classification is the utilization of a set of fuzzy membership functions that determine the degree of membership in the categories young, middle aged and old based on the feature defined in the Age Classification 1 Section. The set of membership functions 91,92,93 shown in Figure 12, are Gaussian functions given by

$$y = e^{\frac{-1}{2\sigma^2}(x-\bar{x})^2} \quad (11)$$

where y is the degree of membership in a sub-set, z is the feature used to determine membership (the PLS age prediction), \bar{z} is the mean or center of the fuzzy sub-set and σ is the standard deviation. However, one skilled in the art will appreciate that the suitable membership function is specific to the application. The mean and standard deviation associated with each of the three categories were determined based on the target population of subjects

Table 1. Parameters of Membership Functions plotted in Figure 12.

Sub-Set Category	Mean (Years)	Standard Deviation (Years)
Young	30	7
Middle Aged	50	10
Old	70	7

in the Experimental Data Set and are listed in Table 1.

Values for the feature inputs to the membership functions that are unusually high or low fall outside that expected range of the sub-sets and are assigned low membership values. This information is used to indicate that the subject's apparent age is outside of the previously examined population and is used for outlier analysis. For the current implementation, when $y < 0.1$ for all sub-sets, the prediction is assigned a low confidence level.

The resulting class memberships are suitable for use in categorization for blood analyte prediction as described in the parent application to the current application: An intelligent system for noninvasive blood analyte prediction, S.

Malin, T. Ruchti, U.S. Patent Application Serial No. 09/359,191, filed July 22, 1999. The membership functions described have been designed for a specific population of subjects and cannot be generalized to all potential individuals. The invention, nevertheless, is directed to the arbitrary use of membership functions to assign a degree of membership in a given class to a subject for blood analyte prediction.

Although the invention is described herein with reference to the preferred embodiment, one skilled in the art will readily appreciate that other applications may be substituted for those set forth herein without departing from the spirit and scope of the present invention. Accordingly, the invention should only be limited by the Claims included below.

CLAIMS

- 5 1. A method for non-invasively determining the apparent age of a tissue sample *in vivo* comprising the steps of:
- measuring the NIR absorption spectrum of said tissue sample;
- detecting outliers, wherein said outliers are invalid measurements caused by spectral variation due to any of instrument malfunction, poor
- 10 sampling, and subjects outside of said calibration set; and
- preprocessing, wherein said preprocessing step includes one or more transformations that attenuate noise and instrumental variation without affecting the signal of interest, including wavelength selection, scaling, normalization, smoothing, derivatives, and filtering; and
- 15 estimating the apparent age of said tissue sample based on said NIR absorption spectrum.
2. The method of Claim 1, wherein said NIR region comprises a range of about 700 to about 2500nm.
- 20
3. The method of Claim 1, wherein said spectrum is denoted by a vector $m \in \mathfrak{R}^N$ of absorbance values pertaining to a set of N wavelengths $\lambda \in \mathfrak{R}^N$.
4. The method of Claim 1, wherein said outlier detection step employs
- 25 principal components analysis and residual analysis to detect spectral outliers.

5. The method of Claim 4, wherein said outlier detection step further comprises the steps of:

projecting a spectrum m onto a plurality of eigenvectors, contained in a matrix o , said matrix o being previously developed through principal components analysis of said calibration set, where

$$xpc_o = \sum_{k=1}^7 mo_k$$

and where o_k is the k^{th} column of the matrix o ;

determining the residual q , according to

$$q = m - xpc_o o^T$$

10

;

comparing said residual q to three times the standard deviation of the residual of said calibration set; and

reporting said sample as an outlier if q is greater.

15 6. The method of Claim 1, wherein said preprocessed measurements are denoted by a vector $x \in \mathfrak{R}^N$, and wherein said vector is determined according to

$$x = h(\lambda, m)$$

and wherein a preprocessing function is $h: \mathfrak{R}^{Nx2} \rightarrow \mathfrak{R}^N$.

20

7. The method of Claim 1, wherein said age estimation step further comprises the step of:

57. The apparatus of Claim 41, wherein said means for analysis comprises a digital processor programmed to perform an age estimation procedure;

wherein said digital value is passed to said relative age estimation procedure and whereby a relative age estimation is performed.

5

58. In an apparatus for non-invasively estimating the relative age of a subject, an optical interface comprising:

means for transmitting NIR energy towards a target measurement site on a subject;

10 means for collecting NIR energy reflected from said measurement site.

59. The optical interface of Claim 58, wherein said transmission means is a light pipe, a fiber-optic probe, a lens system, or a light-detecting mirror.

15 60. The optical interface of Claim 58, wherein said energy collecting means comprises at least one staring optical detector.

61. The optical interface of Claim 58, wherein said energy collecting means comprises at least one fiber-optic probe.

20

62. The optical interface of Claim 58, wherein said optical interface is positioned at optimally determined distances from said target measurement site.

25 63. The optical interface of Claim 62, wherein said optimal distances are specified according to layer of the skin tissue to be sampled.

providing a calibration model to map said preprocessed spectrum through a mapping to an estimate of age.

8. The method of Claim 7, wherein said calibration model comprises NIR
5 tissue measurements and chronological ages of an exemplary subject population.

9. The method of Claim 7, wherein said mapping is linear.

10 10. The method of Claim 7, wherein said age estimate is determined

$$\hat{y} = \sum_{k=1}^N w_{c,k} x_k$$

according to

;

given the preprocessed spectrum x , and the calibration model w_c ,

\hat{y}

where $w_{c,k}$ is the k^{th} element of w_c and \hat{y} is the age estimate.

15

11. The method of Claim 10, wherein said calibration model employs factor analysis to decompose a high-dimensional (redundant) data set comprising absorbance, intensity or reflectance measurements at a plurality of wavelengths to significant factors representing the majority of variation within
20 said data set; and

wherein said calibration model includes factors that capture variation in said spectra correlated with variation in subject age.

12. The method of Claim 10, further comprising the steps of;
projecting said samples into a resulting factor space to produce a set of
scores for each sample; and
applying multiple linear regression to model the relationship between
5 said scores and apparent age of said subject.
13. The method of Claim 7, wherein said mapping is non-linear.
14. The method of Claim 13, wherein said non-linear mapping is specified
10 through any of artificial neural networks and non-linear partial least squares
regression.
15. A method for non-invasively determining the apparent age of a tissue
sample *in vivo* comprising the steps of:
15 providing a calibration set of exemplary measurements;
measuring the NIR absorption spectrum of said tissue sample;
detecting outliers, wherein said outliers are invalid measurements
caused by spectral variation due to any of instrument malfunction, poor
sampling, and subjects outside of said calibration set; and
20 estimating the apparent age of said tissue sample based on said NIR
absorption spectrum.
16. A method of classifying a subject according to age based on
noninvasive NIR measurements, comprising the steps of:
25 providing a calibration set of exemplary measurements;
measuring the NIR absorption spectrum of said tissue sample;

detecting outliers, wherein said outliers are invalid measurements caused by spectral variation due to any of instrument malfunction, poor sampling, and subjects outside of said calibration set;

preprocessing, wherein said preprocessing step includes at least one
5 transformation that attenuates noise and instrumental variation without affecting the signal of interest, including wavelength selection, scaling, normalization, smoothing, derivatives, and filtering,

extracting features, whereby factors of measurements relevant to age classification are determined; and

10 classifying said sample according to predefined age groups.

17. The method of Claim 16, wherein said feature extraction step comprises any mathematical transformation that enhances a quality or aspect of said sample measurement for interpretation to represent concisely the
15 properties and characteristics of the tissue measurement site for age classification.

18. The method of Claim 17, wherein said feature extraction step employs scores from factor analysis.

20

19. The method of Claim 17, wherein said feature extraction step employs partial least squares regression.

20. The method of Claim 17, wherein said features are represented in a

$$z = f(\lambda, x)$$

vector, $z \in \mathcal{R}^M$ that is determined from a preprocessed measurement through:

where $f: \mathcal{R}^n \rightarrow \mathcal{R}^m$ is a mapping from a measurement space to a feature

5 space, wherein decomposing $f(\bullet)$ yields specific transformations, $f(\bullet): \mathcal{R}^n \rightarrow \mathcal{R}^m$,
for determining a specific feature, wherein the dimension M_i indicates whether
an i^{th} feature is a scalar or a vector and an aggregation of all features is the
vector z , and wherein a feature exhibits a certain structure indicative of an
underlying physical phenomenon when said feature is represented as a vector
10 or pattern.

21. The method of Claim 20, wherein individual features are divided into
two categories comprising:

abstract features that do not necessarily have a specific interpretation
15 related to a physical system; and

simple features that are derived from an *a priori* understanding of a
sample and that can be related directly to a physical phenomenon.

22. The method of Claim 21, wherein spectral variations due to changes in
20 dermal thickness corresponding with age are extracted and enhanced, and
wherein said variations serve as a feature for age classification and
measurement of tissue properties.

23. The method of Claim 18, further comprising the step of:

25 employing factor-based methods to build a model capable of
representing variation in a measured absorbance spectrum related to subject

age, wherein projection of a measured absorbance spectrum onto said model constitutes a feature that represents spectral variation related to subject age.

24. The method of Claim 16, wherein said classification step further
5 comprises the steps of:

measuring the similarity of at least one feature to predefined age categories; and

assigning membership to one of said predefined categories.

10 25. The method of Claim 24, wherein said assigning step uses mutually exclusive classes and assigns each sample to one class.

26. The method of Claim 24, wherein said assigning step uses a fuzzy classification system that allows class membership in more than one class
15 simultaneously.

27. The method of Claim 25, wherein said assigning step further comprises the steps of:

mapping said sample to one of said predefined classes; and

20 applying a decision rule to assign class membership.

28. The method of Claim 27, wherein said mapping step is given by:

$$L = f(z)$$

25 where L is a scalar that measures distance of a sample from the

predefined age categories.

29. The method of Claim 28, wherein said age categories are "old" and young" and where L_{old} corresponds to a representative value for said "old" class and L_{young} corresponds to a representative value for said "young class"; and wherein said class assignment is based on the closeness of L to L_{old} and L_{young} .

30. The method of Claim 29, wherein a distance d_{old} of L to L_{old} is measured by

$$d_{old} = |L_{old} - L|,$$

and wherein a distance d_{young} of L to L_{young} is measured by

$$d_{young} = |L_{young} - L|.$$

31. The method of Claim 29, wherein said decision rule is:
if $d_{old} < d_{young}$, then the apparent age of the sample is classified as "old;" and
if $d_{old} \geq d_{young}$, then the apparent age of the sample is classified as "young."

32. The method of Claim 27, wherein limits for said mapping and decision rule are determined from a calibration set of exemplary measurements and corresponding apparent age reference values through a classification calibration procedure.

33. The method of Claim 32, wherein said classification calibration procedure comprises any of linear Discriminant analysis, SIMCA, k nearest neighbor, fuzzy classification and artificial neural networks.

5 34. The method of Claim 26, wherein class membership is defined by a continuum of grades, and wherein a set of membership functions map a feature space into an interval [0,1] for each class and wherein an assigned grade represents a degree of class membership, and wherein a grade of "1" represents the highest degree of class membership.

10

35. The method of Claim 34, wherein the mapping from the feature space to a vector of class memberships is given by:

$$c_k = f_k(z)$$

where $k=1,2, \dots P$, and where $f_k(\bullet)$ is the membership of the k^{th}

15

class,

and where $c_k \in [0,1]$ for all k , and where the vector $c \in \mathbb{R}^P$ is the set of class memberships.

36. The method of Claim 35, wherein a membership function is

$$y = e^{\frac{-1}{2\sigma^2}(z-\bar{z})^2}$$

20

represented by

where y is the degree of membership in a fuzzy sub-set, z is the

z

feature used to determine membership, is the center of a fuzzy subset, and σ is the standard deviation.

37. The method of Claim 35, wherein said membership vector provides the
5 degree of class membership in each of said predefined classes.

38. The method of Claim 1, further comprising the step of performing a blood analyte prediction based on said age estimate.

10 39. The method of Claim 15, further comprising the step of performing a blood analyte prediction based on said age estimate.

40. The method of Claim 16, further comprising the step of performing a blood analyte prediction based on said age classification.

15

41. An apparatus for non-invasively estimating the relative age of a subject comprising:

means for generating near infrared (NIR) energy;

means for separating said generated NIR energy into a plurality of

20 wavelength regions;

an optical interface comprising:

means for transmitting said NIR energy from said wavelength

separating means towards a target measurement site on a subject; and

means for collecting NIR energy emanating from said measurement

25 site;

means for detecting said collected energy and converting said collected energy to a voltage;

means for converting said voltage to a digital value; and

means for analyzing said digital value whereby said analysis results in
5 an estimate of said subject's relative age.

42. The apparatus of Claim 41, wherein said energy source transmits light in the wavelength range of about 700nm to 2500nm.

10 43. The apparatus of Claim 42, wherein said energy source is an LED array or a quartz halogen lamp.

44. The apparatus of Claim 41, wherein said wavelength separating means is a monochromator or an interferometer.

15

45. The apparatus of Claim 41, wherein said wavelength separating means comprises successive illumination through an LED array.

46. The apparatus of Claim 41 wherein said transmission means is a light
20 pipe, a fiber-optic probe, a lens system, or a light-directing mirror system.

47. The apparatus of Claim 41, wherein said energy collecting means comprises at least one staring optical detector.

25 48. The apparatus of Claim 41, wherein said energy collecting means comprises at least one fiber-optic probe.

49. The apparatus of Claim 41, wherein said energy detecting means comprises InGaAs detectors.

5 50. The apparatus of Claim 41, wherein said digitizing means is a 16-bit A/D converter.

51. The apparatus of Claim 41, wherein said optical interface is positioned at optimally determined distances from said target measurement site.

10

52. The apparatus of Claim 51, wherein said distances are specified according to layer of the skin tissue to be sampled.

53. The apparatus of Claim 52, wherein said optimal distance is
15 approximately 0 - 300 μ m for the upper dermis.

54. The apparatus of Claim 52, wherein said optimal distance is approximately .3 – 3mm for the lower dermis.

20 55. The apparatus of Claim 51, wherein a point of illumination is set through any of a focusing lens and a fiber-optic probe.

56. The apparatus of Claim 51, wherein a point of detection is set through any of a staring optical detector or a fiber-optic probe.

25

64. The optical interface of Claim 63, wherein said optimal distance is approximately 0-300 μ m for the upper dermis.

5 65. The optical interface of Claim 63, wherein said optimal distance is approximately .3mm – 3mm for the lower dermis.

66. The optical interface of Claim 58, wherein a point of Illumination is set through any of a focusing lens and a fiber-optic probe.

10

67. The optical interface of Claim 58, wherein a point of detection is set through any of a starring optical detector and a fiber-optic probe

15

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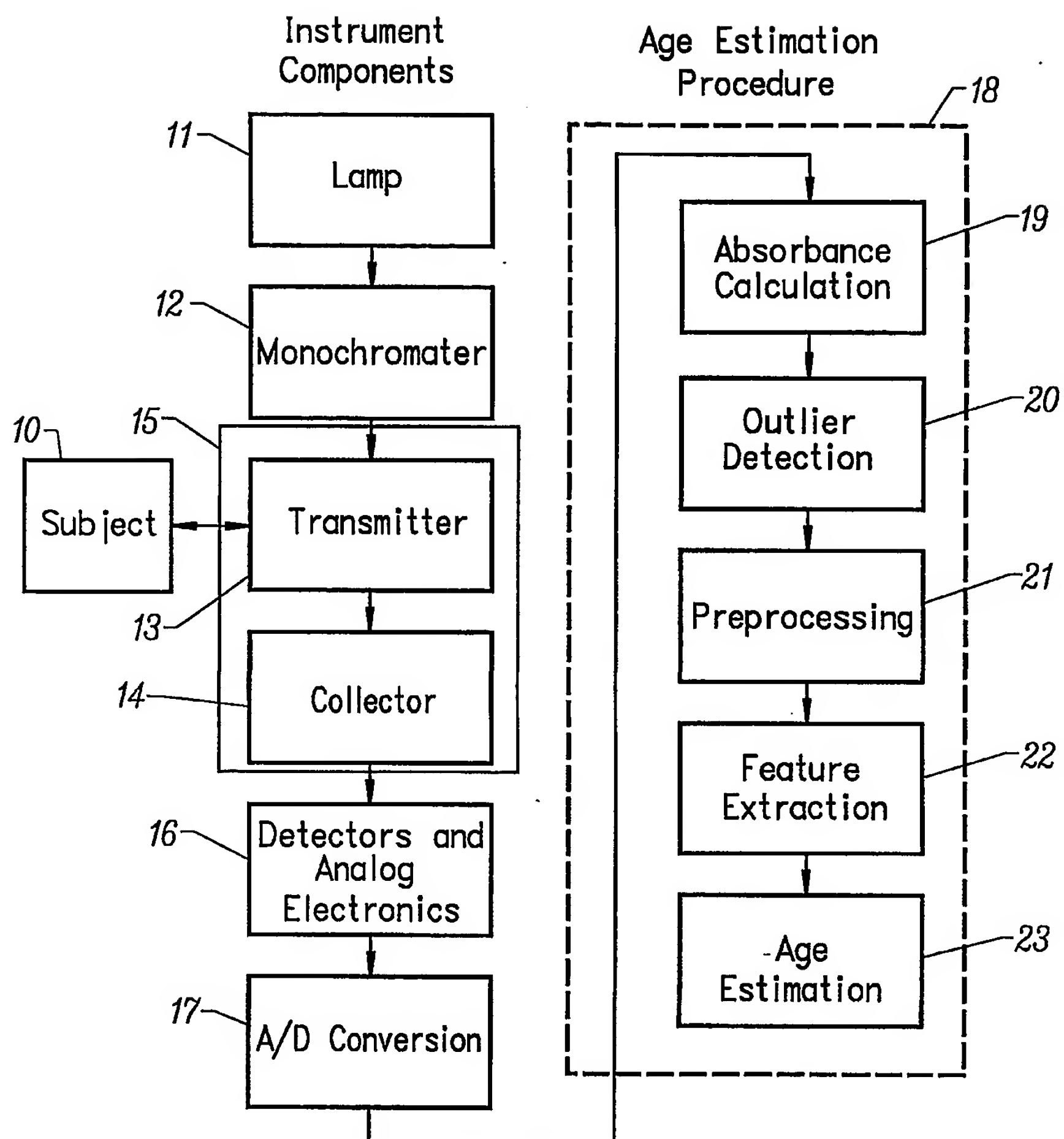
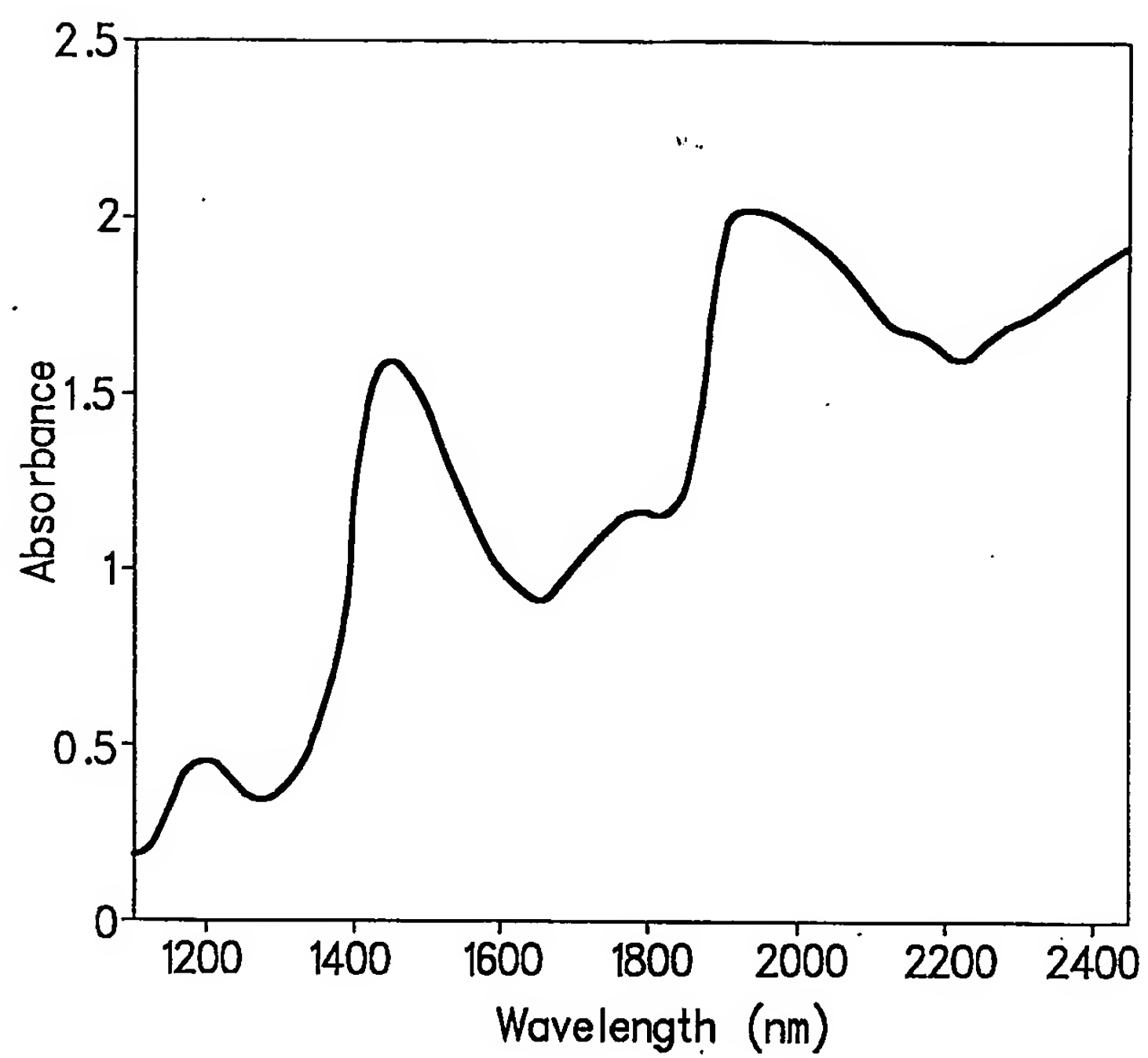


FIG. 1

2/10

*FIG. 2*

3/10

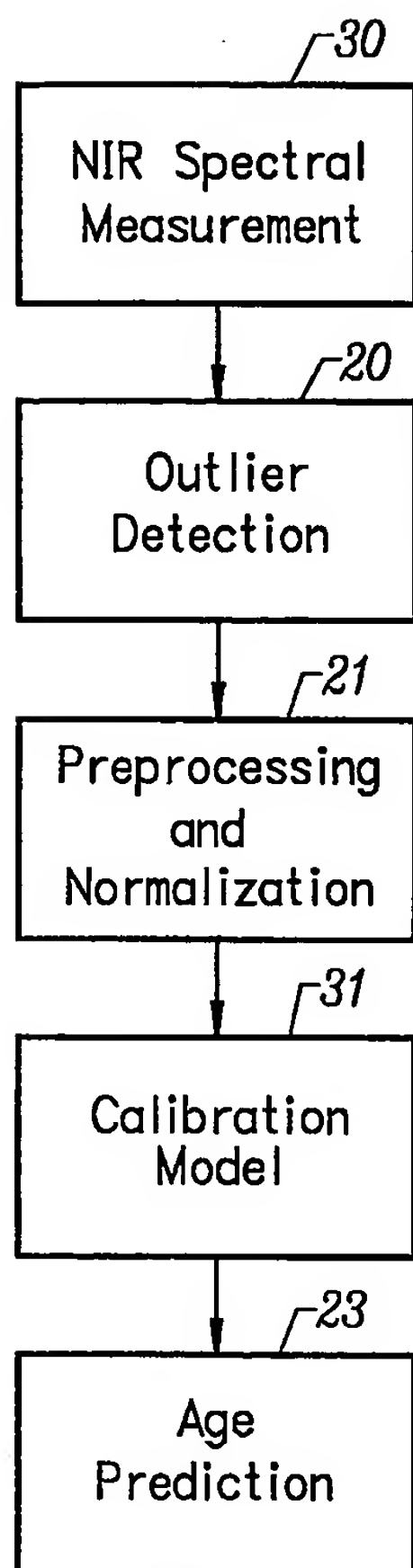


FIG. 3

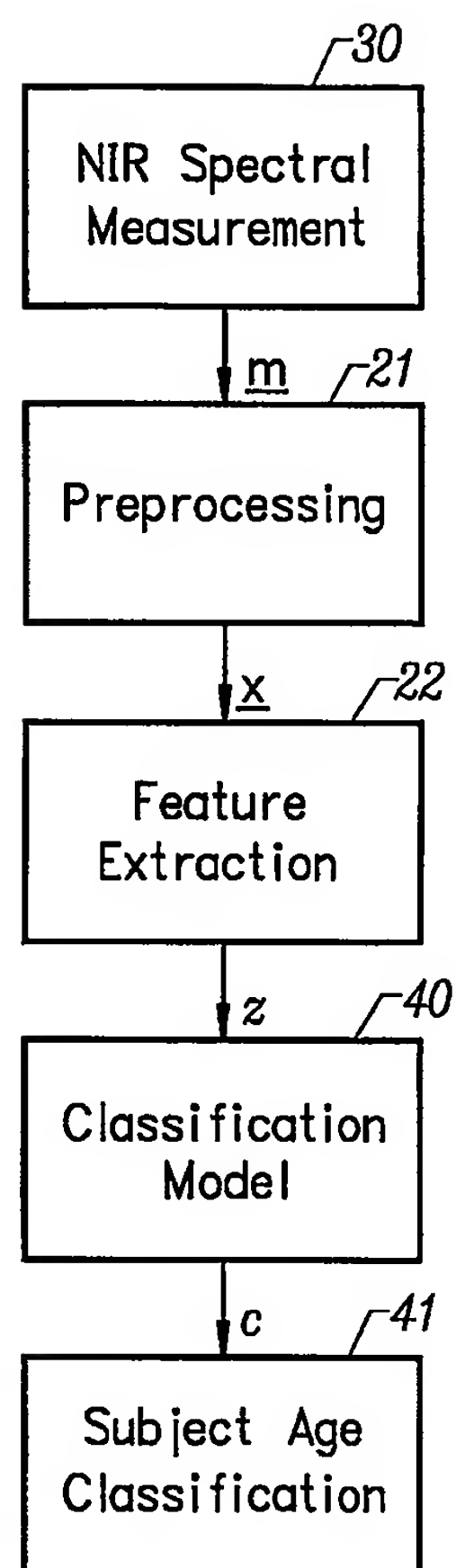
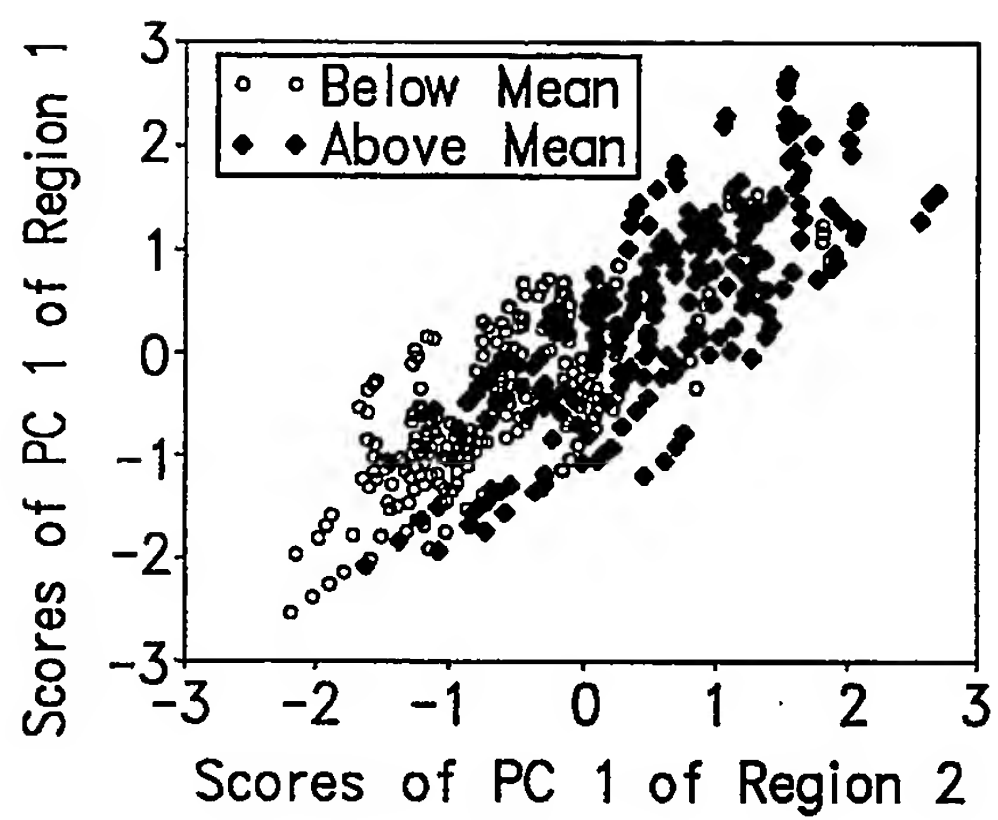
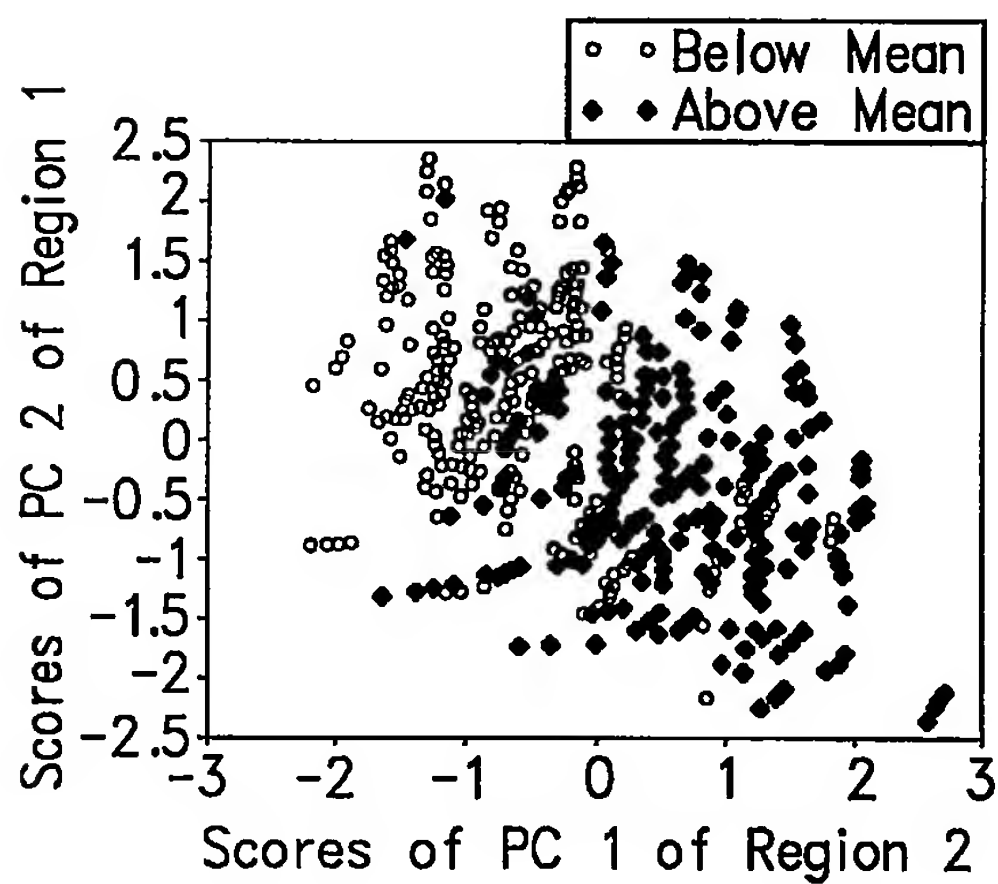
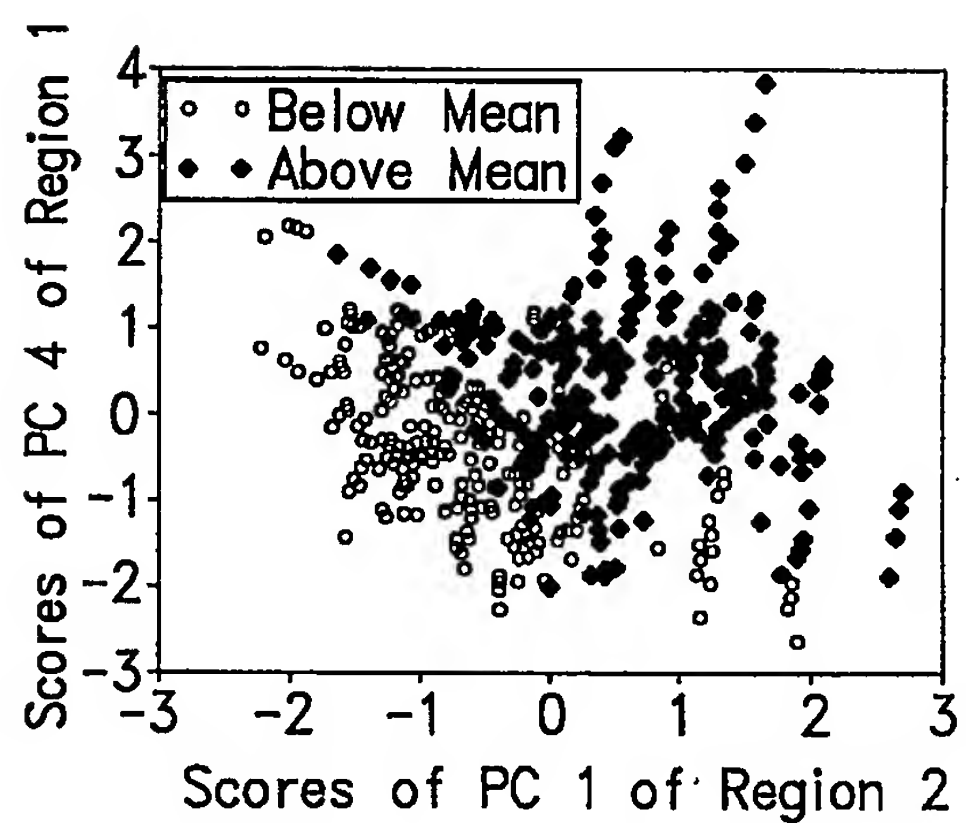
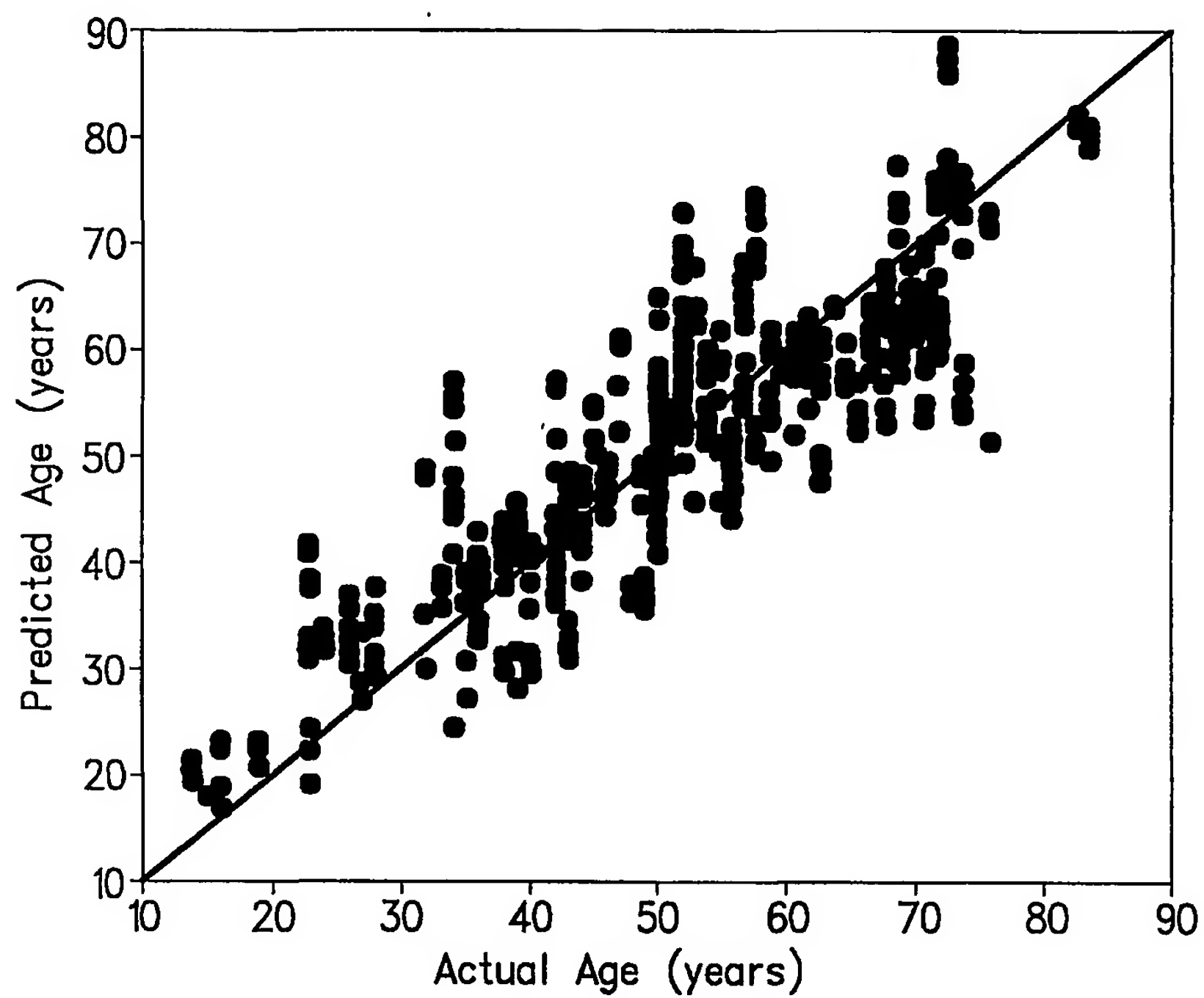


FIG. 4

4/10

*FIG. 5A**FIG. 5B**FIG. 5C*

5/10

*FIG. 6*

6/10

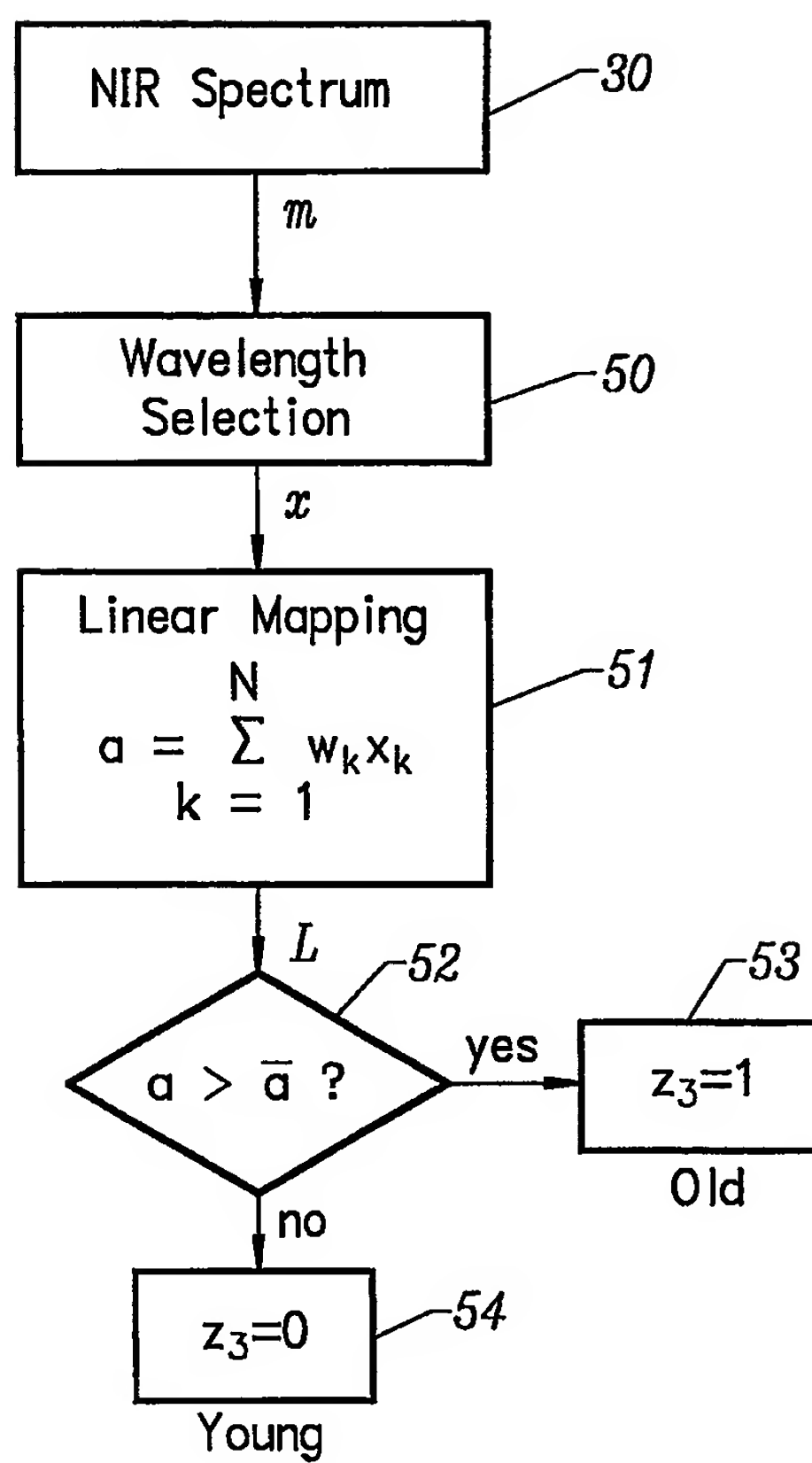


FIG. 7

7/10

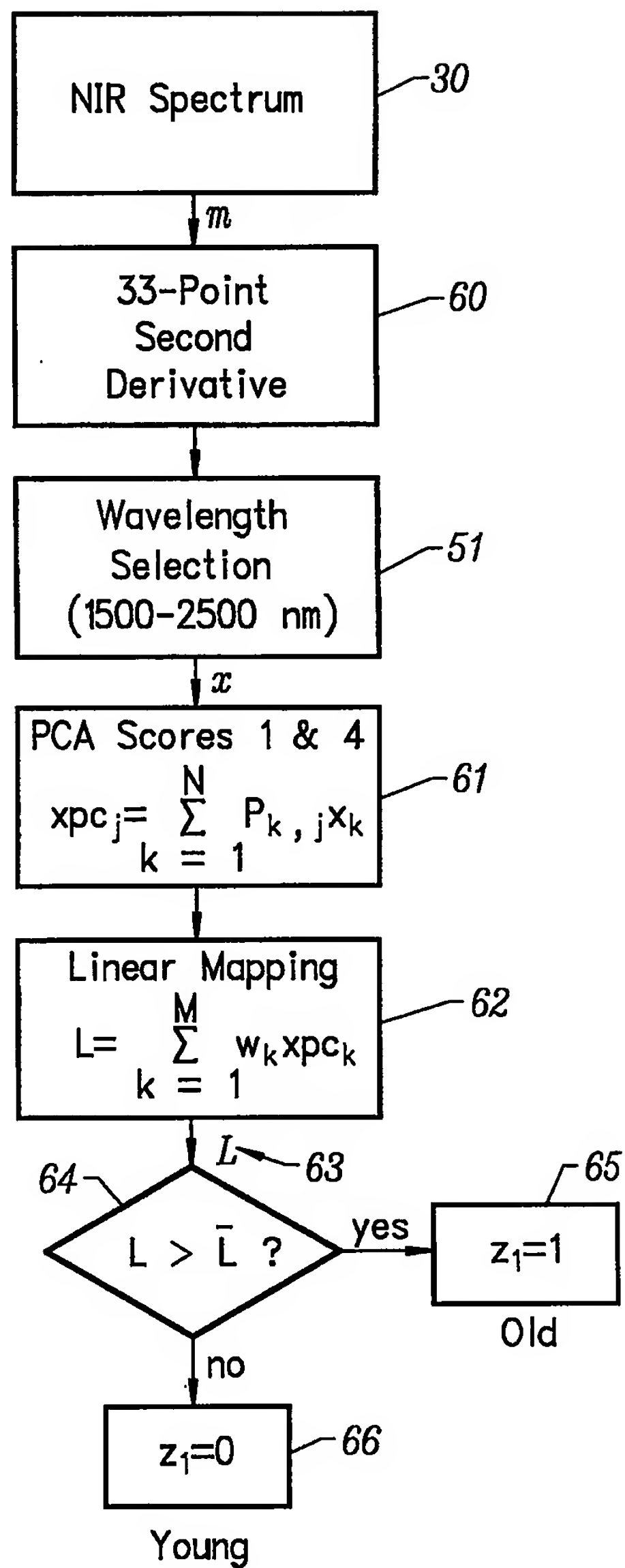


FIG. 8

8/10

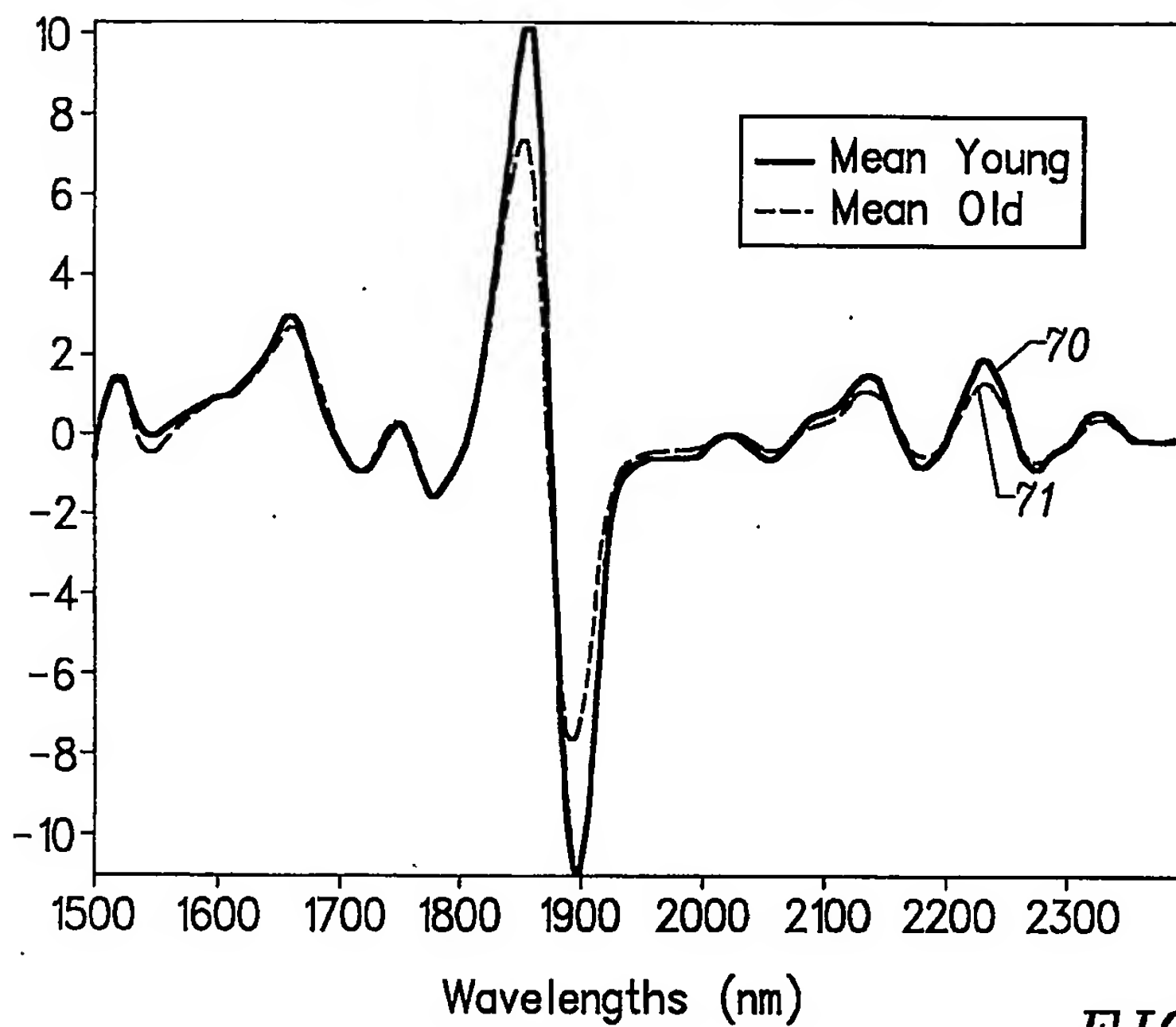
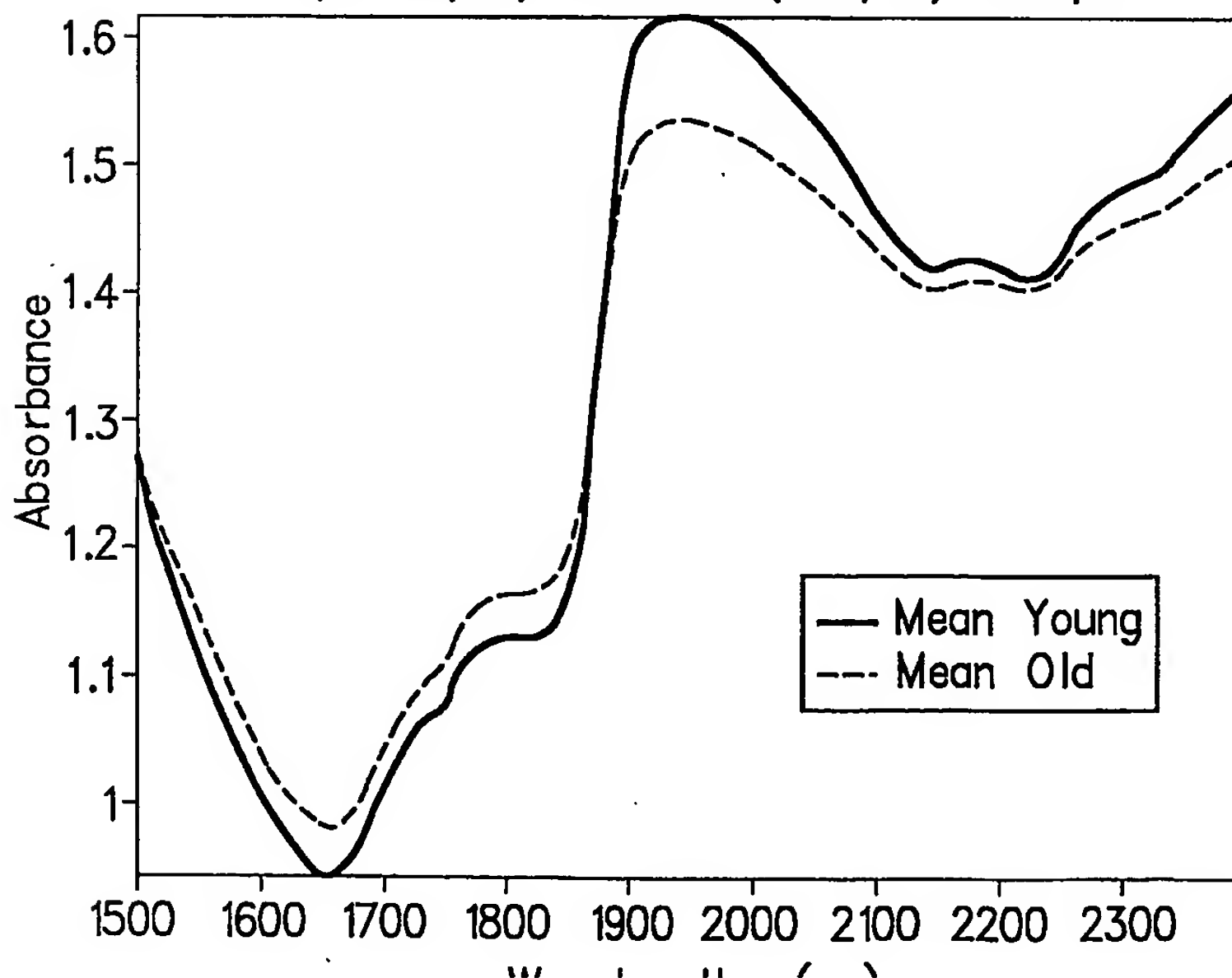
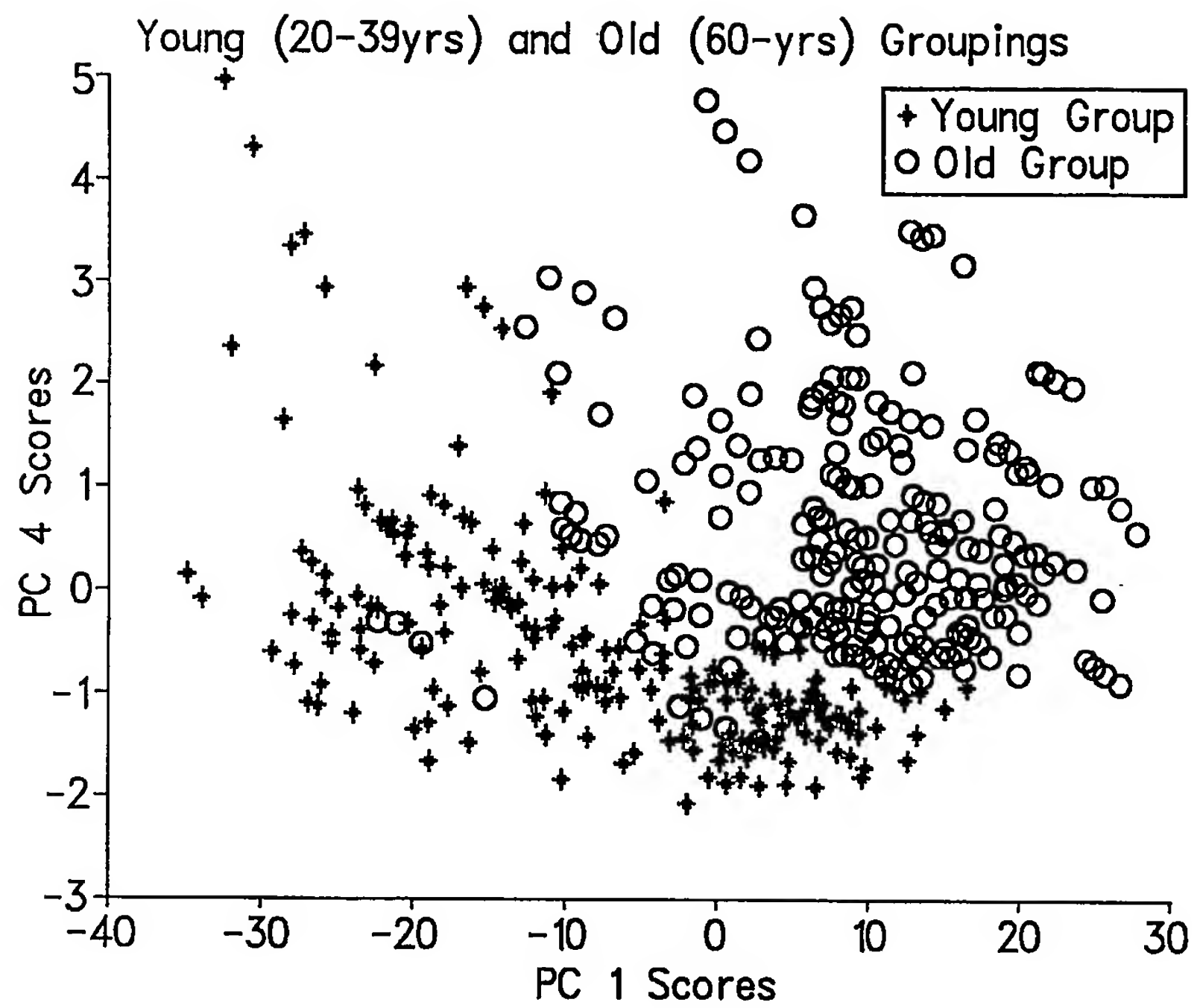
Mean Second Derivative Spectra of Young
(20-39yrs) and Old (60+yrs)

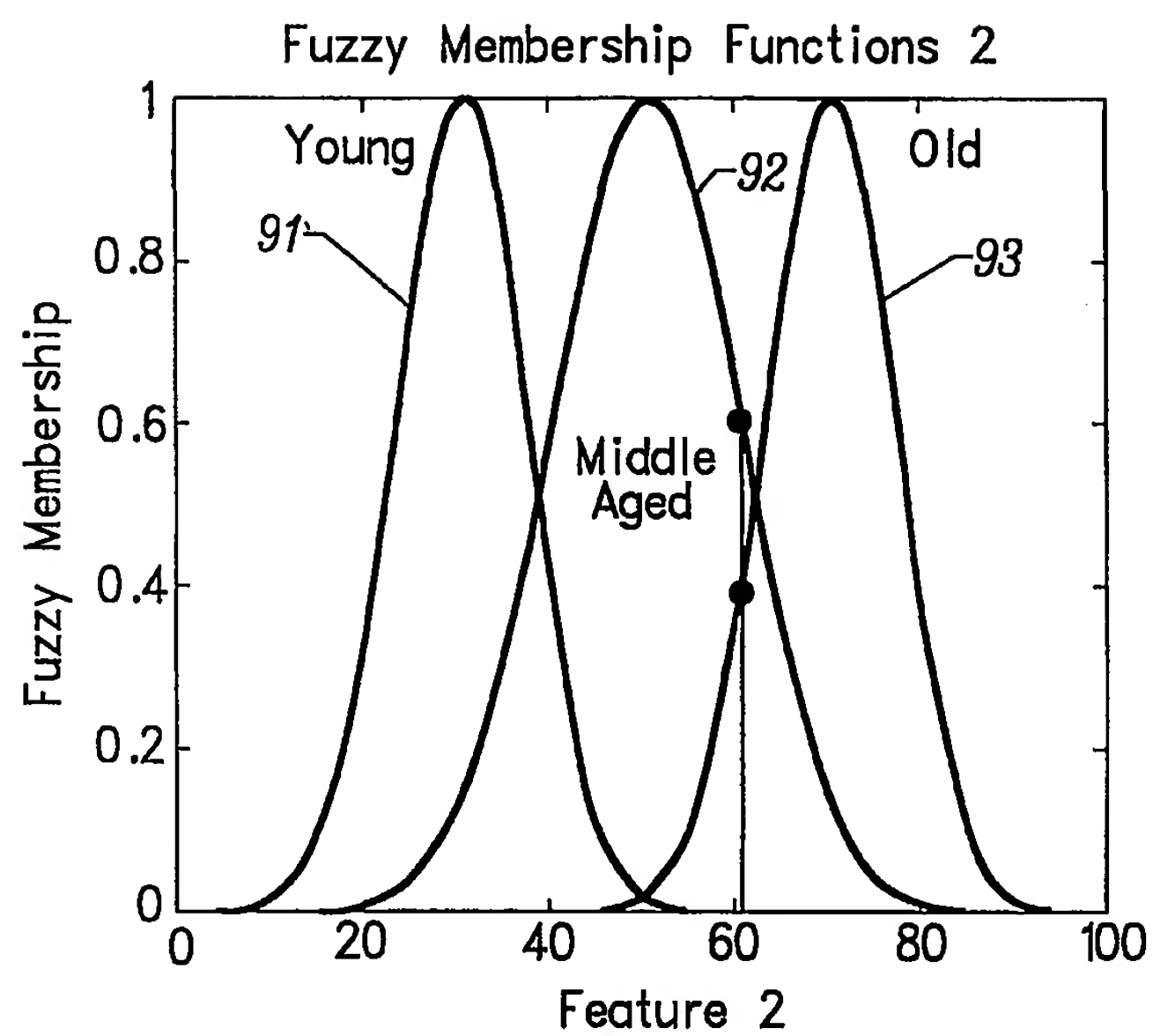
FIG. 9

Mean Spectra of Young
(20-39yrs) and Old (60+yrs) Groups

9/10

*FIG. 11*

10/10

*FIG. 12*

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/33754

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/35 A61B5/103

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAES T ET AL: "COMPARISON OF MULTIVARIATE CALIBRATION AND DISCRIMINANT ANALYSIS IN EVALUATING NIR SPECTROSCOPY FOR DETERMINATION OF MEAT TENDERNESS" APPLIED SPECTROSCOPY, US, THE SOCIETY FOR APPLIED SPECTROSCOPY. BALTIMORE, vol. 51, no. 3, 1 March 1997 (1997-03-01), pages 350-357, XP000698671 ISSN: 0003-7028	58-60
A	page 353, right-hand column, line 10 - line 19 ----- -/--	1,15,16, 41,61, 66,67

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

5 June 2001

Date of mailing of the international search report

11/06/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Verdoodt, E

INTERNATIONAL SEARCH REPORT

I Application No
PCI/US 00/33754

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BROOKS A ET AL: "NEW METHOD FOR INVESTIGATIONS OF NORMAL HUMAN SKIN SURFACES IN VIVO USING FIBER-OPTIC EVANESCENT WAVE FOURIER TRANSFORM INFRARED SPECTROSCOPY (FRE-FTIR)" SURFACE AND INTERFACE ANALYSIS, HEYDEN AND SON, LONDON, GB, vol. 27, no. 4, April 1999 (1999-04), pages 221-229, XP000989820 ISSN: 0142-2421 page 221, right-hand column, line 15 - line 23	1,15,16, 41,58
A	US 5 931 779 A (BURNS DAVID H ET AL) 3 August 1999 (1999-08-03) column 9, line 66 - column 10, line 8 column 19, line 16 - line 46	1,15,16, 41,58
A	WO 97 36540 A (FLORIDA INST OF TECHNOLOGY) 9 October 1997 (1997-10-09) page 14, paragraph 1 page 15, last paragraph - page 16, line 1	1,15,16, 41,58
A	QUAN M B ET AL: "NON-INVASIVE IN VIVO TECHNIQUES TO DIFFERENTIATE PHOTODAMAGE AND AGEING IN HUMAN SKIN" ACTA DERMATO-VENEREOLOGICA, XX, XX, vol. 77, 1997, pages 416-419, XP000989966 ISSN: 0001-5555 cited in the application the whole document	1,15,16, 41,58
A	GELADI P ET AL: "LINEARIZATION AND SCATTER-CORRECTION FOR NEAR-INFRARED REFLECTANCE SPECTRA OF MEAT" APPLIED SPECTROSCOPY, US, THE SOCIETY FOR APPLIED SPECTROSCOPY. BALTIMORE, vol. 39, no. 3, 1985, pages 491-500, XP000560624 ISSN: 0003-7028 cited in the application the whole document	1,15,16, 41,58

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/33754

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5931779 A	03-08-1999	NONE	
WO 9736540 A	09-10-1997	US 5553616 A	10-09-1996

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a block diagram of an age estimation apparatus and procedure according to the invention;

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Figure 2 is a plot of a typical NIR absorbance spectrum of an *in vivo* sample of skin tissue;

Figure 3 provides a block diagram of a general procedure for predicting
10 subject age based on NIR spectral measurements according to the invention;

Figure 4 provides a block diagram of a classification system for determining the age of a subject based on NIR spectral measurements according to the invention;

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Figure 5 provides a scatter plot of selected principal component scores showing systematic separation by subject age according to the invention;

Figure 6 is a graph of estimated age versus actual chronological age
20 according to the invention;

Figure 7 is a block diagram of a first procedure for classification of subjects into age categories according to the invention;

25 Figure 8 is a block diagram of a second procedure for classification of subjects into age categories according to the invention;